



TRANSMITTAL OF SEQUENCE LISTING AND PRELIMINARY AMENDMENT  
 Application No. 10/023,637  
 Filed December 17, 2001

# AMENDMENTS TO THE SPECIFICATION

Please replace paragraph beginning on line 12, page 89, with the following amended paragraph:

As one illustration of this method, in the case of BVDV in MDBK cells, in a first step, viral RNA is isolated from 140  $\mu$ L of the cell culture supernatant by means of a commercially available column (Viral RNA extraction kit, QiaGen, CA). The viral RNA is then eluted from the column to yield a total volume of 60  $\mu$ L, and subsequently amplified with a quantitative RT-PCR protocol using a suitable primer for the BVDV NADL strain. A quenched fluorescent probe molecule is hybridized to the BVDV DNA, which then undergoes exonucleolytic degradation resulting in a detectable fluorescent signal. Therefore, the RT-PCR amplified DNA was detected in real time by monitoring the presence of fluorescence signals. The TaqMan probe molecule (5' 6-fam-AAATCCTCCTAACAAGCGGGTTCCAGG-tamara 3' [Sequence ID No. [[7]] 1] and primers (sense: 5'-AGCCTTCACTTCTTGCTGATGT-3' [Sequence ID No. [[8]] 2]; and antisense: 5'-TGTTCGCGAAAGCACCAACAG-3' [Sequence ID No. [[9]] 3]) were designed with the aid of the Primer Express software (PE-Applied Biosystems) to be complementary to the BVDV NADL NS5B region. A total of 10  $\mu$ L of RNA was analyzed in a 50  $\mu$ L RT-PCR mixture. Reagents and conditions used in quantitative PCR were purchased from PE-Applied Biosystems. The standard curve that was created using the undiluted inoculum virus ranged from 6000 plaque forming units (PFU) to 0.6 PFU per RT-PCR mixture. A linear range of over 4-logs was routinely obtained.

Please enter the attached Sequence Listing into the Specification.

Attachments: Nucleotide Sequence Listing in written form (2 pp.)  
 Diskette containing the Sequence Listing in computer readable form